REGNERATION OF THE KIDNEY IN THE RED-BELLIED TOAD (BOMBINA BOMBINA)

A. G. Babaeva

Laboratory of Growth and Development (Head, Professor L. D. Liozner),
Institute of Experimental Biology (Director, Professor I. N. Maiskii),
of the AMN SSSR, Moscow

Presented by Active Member AMN SSSR, A. V. Lebedinskii
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 57, No. 1,
pp. 103-107, January, 1964
Original article submitted January 15, 1964

The regeneration of certain internal organs takes place in a similar manner in amphibians and mammals [3, 4, 5, 7]. The question of the regeneration and compensatory hypertrophy of the kidneys has received little study in its comparative aspect. The principal object for studying regenerative processes has so far been the mammalian kidney, and few investigations have been made of the kidney of other classes of vertebrates, especially amphibians. V. V. Lavrenko [2] showed that regeneration of the kidney of the grass frog could take place after burning with a red-hot needle. The ability of the kidney to undergo compensatory hypertrophy and regeneration after removal of a considerable volume of tissue in experiments on axolotls and pond frogs was demonstrated by I. A. Knorre [1]. She showed that both these processes, as in mammals, are accompanied by hypertrophy of the structural units of the kidney. However, this worker had available only a small number of animals (1-2 for each period of sacrifice), so that the dynamics of these changes could not be established.

To obtain more accurate data concerning the mode of regeneration and the times of appearance of compensatory hypertrophy, experiments were performed on toads.

EXPERIMENTAL METHOD

Experiments were conducted on 130 adult red-bellied toads (Bombina bombina) of both sexes. One kidney was removed from the animals of group 1, and one whole kidney and $\frac{2}{3}$ of the other from the animals of group 2. In the first and second series of experiments the operation was performed in two stages at an interval of 2-3 months, and in the third series of experiments in one stage. The animals were anesthetized with ether. The animals undergoing operation were given individual tags.

The animals were sacrificed after 1-$5\frac{1}{2}$ months, and the kidneys were weighed on torsion scales, fixed in 10% formalin, and embedded in paraffin wax. Serial sections, 8 μ in thickness, were stained with hematoxylin and eosin. In individual series the number of renal corpuscles was counted in every 16th section, and by means of a drawing apparatus measurements were made of the diameter of the renal corpuscle, the vascular glomerulus (50-60 renal corpuscles: magnification, ocular 3, objective 15), the initial portion of the tubules, and the lumen of the tubules (50 transverse sections of the tubules: magnification, ocular 20, objective 20). Next, in 5-6 cases for each series the area occupied by a renal corpuscle, and by a tubule and its lumen was calculated from the formula of an elipse: $C = \frac{\Pi D\mu \cdot d\mu}{4}$.

In the same series the mitotic activity of the tubular epithelium was determined by examining 10,000 cells under the binocular microscope (ocular 7, objective 90). The mitotic coefficient was calculated per 1000 cells. All the numerical results were subjected to statistical analysis by the Fisher-Student method.

EXPERIMENTAL RESULTS

Compensatory hypertrophy of the kidney (first group). One month after nephrectomy (Table 1) the changes in the absolute weight of the remaining kidney were within the limits of variation of the weight of the kidney in the control animals. Two months after the operation the absolute weight of the kidney of the experimental animals still showed no significant difference from the weight of the kidney in the control animals. Five months after the operation the weight of the residual kidney showed a marked increase, but the difference between the experimental and control weights was not statistically significant (P = 0.08).

TABLE 1. Changes in Individual Indices During Compensatory Hypertrophy of the Kidney

Group of animals	Number of animals	Weight of kidney		Ratio between wt. of residual kidney and weight of both kidneys in controls		Number of glomeruli in kidney	Area (in μ^2)	
		in mg	As percentage of body weight	Absolute weight	Relative weight		Of renal corpuscle	Of vascular glomerulus
Experimental (1 month after operation)	12	18	0.37	45	57.8			
Control	12	20	0.32					
Experimental (2 months after operation)	9	15.7	0.44	52	68.7	199	7111	4280
Control	14	15.2	0.32			236	5898	3601
P						0.15	0.15	0.15
Experimental (3 months after operation)	6	15.5	0.45	47	66.1			
Control	5	16.5	0.34					
Experimental (5 months after operation	7	24.2	0.58	59	70.7			
Control	10	20.5	0.38					
Experimental (5½ months after operation	14	19.1	0.46	60	76.3	208	8130	5219
Control	16	15.8	0.32			236	6576	4174
P						0.17	0.05	0.06

* The weight of one kidney is given for the control animals.

TABLE 2. Changes in Individual Indices During Regeneration of the Kidney

Group of animals	Number of animals	Weight of regenerating kidney	Weight of removed part of the kidney (in mg)	Weight of regenerating kidney: In mg	Weight of regenerating kidney: As a percentage of body weight	Number of renal corpuscles in one kidney	Mitotic coefficient (in %)	Area (in μ^2): Of a renal corpuscle	Area (in μ^2): Of a vascular glomerulus
Experimental (removal of one kidney followed 3 months later by removal of ⅔ of the other)	8	19.2	15.1	18	0.32				
Control	10	—	—	20.5	0.38				
Experimental (removal of one kidney followed 2 months later by removal of ⅔ of the other)	7	18.1	16.6	18.1	0.31	94	0.1	9717	5994
P						0.001		0.000	0.000
Experimental (one-stage removal of one whole kidney and ⅔ of the other)	15	15.3	9.4	13.1	0.28	112	0.24	11740	6742
P						0.000	0.000	0.002	0.008
Control	16	—	—	15.8	0.32	236	0.06	6576	4174

The weight of one kidney is given for the control animals.

In the next season the experiments were repeated on a larger number of animals. Three months after nephrectomy the weight of the residual kidney was actually less than the weight of one kidney in the control animals. After 5½ months the weight of the kidney of the experimental animals was greater, but the difference was still only approaching significant (P = 0.008). The absence of definite hypertrophy in terms of absolute weight was due to the varied response of the animals to nephrectomy. In 8 of the 14 experimental animals the weight of the residual kidney was increased, in 3 animals the weight of the kidney was essentially unchanged, and in 3 animals it showed a marked fall, so that the results canceled each other.

It may be concluded that 5-5½ months after the operation, although hypertrophy of the kidney was observed in the experimental animals, it was much less in degree than in mammals. This was shown by the fact that the weight of the kidney did not increase in all the toads.

The presence of hypertrophy of the kidney was also demonstrated by measurements of the area of the renal corpuscle. Two months after the operation the area of the corpuscle and of the vascular glomerulus very slightly exceeded their area in the control animals; the difference was not significant (P = 0.15). After 5½ months the difference between the area of the renal corpuscle in the experimental and control animals was close to significant (P = 0.05).

The mitotic activity at this period was indistinguishable from normal (0.7%). The number of renal corpuscles in the course of the whole experiment remained unchanged, the same as in the control animals.

A noteworthy finding was the slow development of the compensatory reaction in the toad's kidneys. In mammals, hypertrophy of the kidney was observed after only one week and reached its maximum after 40 days [8]. In the toad, however, hypertrophy of the kidney was not observed until 5 months after nephrectomy.

A considerable increase in the relative weight of the residual kidney over the weight of the kidney in the control animals was observed, and this increase was not significant only during the first month. At all subsequent times the relative weight of the solitary kidney

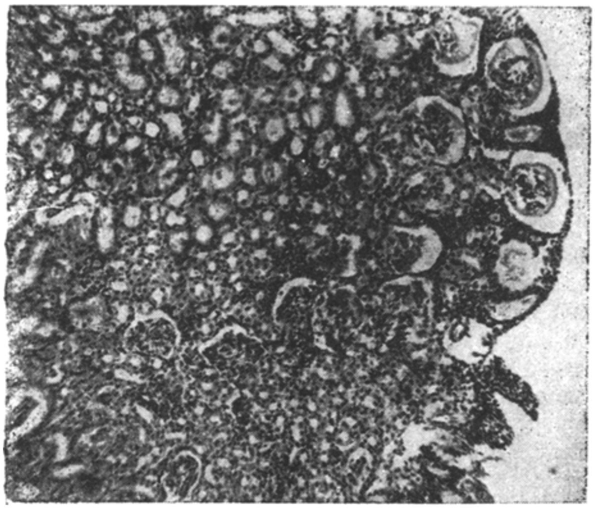

Fig. 1. Renal corpulscles and tubules of a control toad. Photomicrograph. Ocular 6.5×. Stained with hematoxylin-eosin.

of the experimental animals was significantly greater than the relative weight of one kidney of the control animals. The reason for this evidently was that the experimental toads gained weight more slowly than the controls.

Regeneration of the kidney (second group). The object of the first two series of experiments in this group was to determine whether the hypertrophied kidney is capable of regeneration. Since, however, practically no hypertrophy was observed during the first 2 or 3 months, the results of this series of experiments were used to compare the course of regeneration after a single-stage removal of five-sixths of the renal tissue (third series) and after a two-stage removal of the same amount of tissue with a considerable interval between the operations. The experiments were also performed on two consecutive seasons. In the first series of experiments one kidney was removed, followed 2 months later by ⅔ of the remaining kidney. In the second series of experiments ⅔ of the remaining kidney was removed 3 months after removal of the first kidney. In both series the animals were sacrificed 5½ months after the first operation. In these series of experiments the residual portion of kidney (⅓) regenerated, i.e., its weight was restored. Both the absolute and the relative weight of the kidney attained, or nearly attained the weight of one kidney in the control animals. The length of the organ changed relatively little, and no outgrowth of tissue from the wound surface was observed, but the organ mainly grew in width and thickness. In the second series of experiments the restoration of weight was more complete than in the first, presumably on account of the longer period of regeneration in the animals of the second series. Counts of the number of renal corpuscles and measurement of their area showed that restoration of the weight of the kidney in toads takes place, not by an increase in the number of structural units, but a statistically significant increase in their size—by 44% (Table 2). The mean number of renal corpuscles in the regenerating kidney (94) was only slightly greater than their number in the residual ⅓ of the kidney (78).

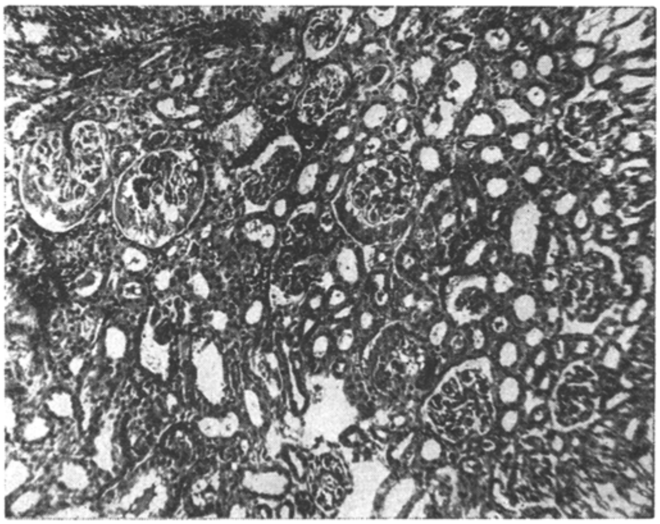

Fig. 2. Enlargement of renal corpulscles and tubules in the regenerating kidney. Photomicrograph. Ocular 6.5×, objective 21×. Stained with hematoxylin-eosin.

After the one-stage removal of one whole kidney and ⅔ of the second kidney (third series of experiments) a considerable degree of restoration of the weight took place, although it was less complete than in the preceding experiment. The difference between the absolute weight of the kidney in the second and third series of experiments was significant ($P=0.004$). The measurements of the renal corpuscles were increased by 77%. The number of renal corpuscles in the regenerating kidney was much smaller than in the control animals, i.e., the formation of new nephrons could not be determined. To obtain a more complete picture of the regeneration process, measurements were made of the area of a tubule, of its lumen, and of the tubular epithelium. These showed that during regeneration after one-stage removal of five-sixths of the kidney, hypertrophy of all the components of the tubules took place very intensively. The area occupied by a tubule, its lumen, and its epithelium, amounted to 205, 219, and 201% respectively of the area in the control animals (Figs. 1 and 2).

The investigation of the mitotic activity of the tubular epithelium of the kidneys in the animals of the third series of experiments showed that the process of regeneration was not yet complete at this time, for the mitotic coefficient was 4 times greater than in the controls ($P = 0.000$) and 2.5 times greater than in the second series. It should, however, be noted that the mitotic activity was determined during the daytime without taking the diurnal rhythm into consideration.

Hence, the kidney of the red-bellied toad is capable of regeneration, which takes place, as in mammals [6], by regeneration hypertrophy. Regeneration hypertrophy of the kidneys in amphibians is also characterized by hypertrophy of the nephrons (the area of the tubules is roughly doubled, and the area of the renal corpuscles is increased by roughly 50%) and is unaccompanied by any significant increase in their number. The process of hypertrophy of the structural units of the organ is much more intensive in regeneration than in compensatory hypertrophy, presumably on account of the removal of a larger amount of tissue in the regeneration experiments.

SUMMARY

A study was made of compensatory hypertrophy and regeneration of the kidneys in Bombina bombina. Compensatory hypertrophy was manifested only after a long period of time elapsed after the operation–5, 5½ months–and was observed in only 50% of the animals. Compensatory hypertrophy is characterized by some increase of the area of the renal corpuscles. Regeneration occurred following a single stage total excision of one kidney and ⅔ of the second, as well as following excision of ⅔ of the second kidney 2½ and 3½ months after unilateral nephrectomy. Regeneration follows the course of regenerative hypertrophy and is characterized by increase of the weight of the kidney of the area of the renal corpuscles (by 50%) and of all the tubular components (which are doubled in area). This process is more intense than in compensatory hypertrophy.

LITERATURE CITED

1. I. A. Knorre, Effect of function on development and regeneration processes in the renal tissue of certain vertebrates. Author's abstract of candidate dissertation, Moscow (1953).
2. V. V. Lavrenko, Proceedings of the Fifth All-Union Congress of Anatomists, Histologists, and Embryologists [in Russian], p. 503, Leningrad (1951).
3. L. D. Liozner, Byull. éksper. biol., 8, 98 (1961).
4. L. D. Liozner, N. S. Artem'eva, I. V. Markelova, et al., In Book: Regenerative Processes in Vertebrate Animals [in Russian], p. 240, Moscow (1959).
5. L. D. Liozner, N. S. Artem'eva, I. V. Markelova, et al., In Book: Processes of Regeneration and Cell Proliferation [in Russian], p. 109, Moscow (1961).
6. L. D. Liozner and G. G. Samsonidze, In Book: Regeneration of Organs in Mammals [in Russian], p. 267, Moscow (1960).
7. L. K. Romanov, In Book: Regenerative Processes in Vertebrate Animals [in Russian], p. 160, Moscow (1959).
8. T. Addis and W. Lew, J. exp. Med., 71, p. 325 (1940).